

AMENDMENTS TO THE SPECIFICATION UNDER REVISED 37 C.F.R. § 1.121

Please replace the section entitled "Example B Cell Free Inhibition Assay utilizing a Synthetic APP Substrate," spanning from page 127, line 23 to page 128, line 22, with the following:

Example B

Cell Free Inhibition Assay utilizing a Synthetic APP Substrate

A synthetic APP substrate that can be cleaved by beta-secretase and having N-terminal biotin and made fluorescent by the covalent attachment of Oregon green at the Cys residue is used to assay beta-secretase activity in the presence or absence of the inhibitory compounds of the invention. Useful substrates include the following:

Biotin-SEVNLDAEFRC [Oregon green] KK [SEQ ID NO: 1]

Biotin-SEVKMDAEFRC [Oregon green] KK [SEQ ID NO: 2]

Biotin-GLNIKTEEISEISYEVEFRC [Oregon green] KK [SEQ ID NO: 3]

Biotin-ADRGLTTRPGSGLTNIKTEEISEVNLDAEFRC [Oregon green] KK
[SEQ ID NO: 4]

Biotin-FVNQHLCoxGSHLVEALY-LVCoxGERGFFYTPKAC [Oregon green] KK
[SEQ ID NO: 5]

The enzyme (0.1 nanomolar) and test compounds (0.001 - 100 micromolar) are incubated in pre-blocked, low affinity, black plates (384 well) at 37 degrees for 30 minutes. The reaction is initiated by addition of 150 millimolar substrate to a final volume of 30 microliter per well. The final assay conditions are: 0.001 - 100 micromolar compound inhibitor; 0.1 molar sodium acetate (pH 4.5); 150 nanomolar substrate; 0.1 nanomolar soluble beta-secretase; 0.001% Tween 20, and 2% DMSO. The assay mixture is incubated for 3 hours at 37 degrees C, and the reaction is terminated by the addition of a saturating concentration of immunopure streptavidin. After incubation with streptavidin at room temperature for 15 minutes, fluorescence polarization is measured, for example, using a LJL Acquest (Ex485 nm/ Em530 nm). The activity of the beta-secretase enzyme is detected by changes in the fluorescence polarization that occur when the substrate is cleaved by the enzyme. Incubation in the presence or absence of compound inhibitor demonstrates specific inhibition of beta-secretase enzymatic cleavage of its synthetic APP substrate.